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Cyanobacterial biomass: a striking factor to decrease polyaluminium chloride (PACl) coagulation efficiency during a successive bloom

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ABSTRACT

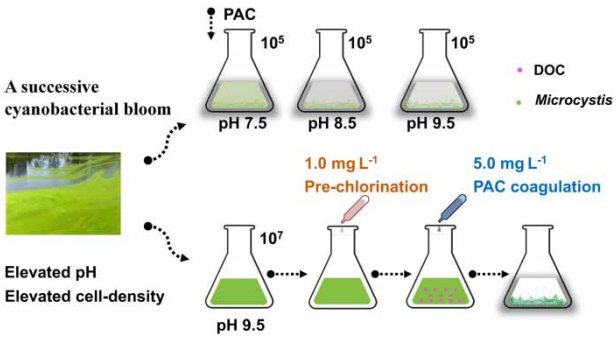
Occurrence of cyanobacterial blooms in source waters challenges water treatment processes. During a successive bloom, typical characteristics of elevated cell-density and pH were observed from development to maintenance stage. However, studies about their influences on the coagulation process have been limited. Here, PACl coagulation experiments were conducted to investigate *Microcystis* removal with varied pH and cell-density. Results showed that PACl coagulation alone was sufficient to remove *Microcystis* with low cell-density (10^5–10^6 cells mL^{-1}), since an elevated pH value (8.5–9.5) can promote PACl coagulation, possibly ascribed to sweeping cells via neutral gelatinous precipitate of alum. Nevertheless, elevated cyanobacterial biomass was a striking factor in decreasing *Microcystis* removal (80%–100%) by PACl coagulation, since its inhibitory effects on the coagulation process could not be offset by *in situ* elevated pH value. Chlorination-assisted (1 mg L^{-1}) coagulation was recommended to treat cyanobacteria-laden source waters with high cell-density of $>10^7$ cells mL^{-1}, as it promoted cyanobacterial removal and achieved the highest removal ratio of DOC and turbidity among these treatments. These findings will provide an important reference for water supplies to choose the proper water treatment process to treat cyanobacteria-laden source waters during a successive bloom.

Key words: cell-density, *Microcystis*, pH values, polyaluminium chloride (PACl) coagulation, pre-chlorination, successive bloom

HIGHLIGHTS

- Elevated pH (8.5–9.5) enhanced cyanobacterial removal by PACl coagulation.
- Elevated cell-density (10^7 cells mL^{-1}) hindered cyanobacterial removal by PACl coagulation.
- Coagulation alone was sufficient to remove cyanobacteria of 10^5–10^6 cells mL^{-1}.
- Pre-chlorination was necessary to treat elevated cyanobacterial biomass of 10^7 cells mL^{-1}.

GRAPHICAL ABSTRACT



INTRODUCTION

Lakes and reservoirs are important water resources for urban water supply systems, but global climate change and eutrophication will enhance the outbreak frequency of harmful cyanobacterial blooms in source waters (O'Neil *et al.* 2012). Recently, ecologists have proposed the concept of 'a successive bloom' according to the long-term observation of cyanobacterial blooms in several lakes (Tang *et al.* 2018; Wilhelm *et al.* 2020). Generally, cyanobacterial blooms (e.g., in Taihu lake) can be classified into three stages: development, maintenance and decay stages (Tang *et al.* 2018). From the development to maintenance stage, the cell-density of cyanobacteria is increasing (Tang *et al.* 2018). The pH values of natural waters also increased, as cyanobacteria can utilize CO_2 as carbon source to biosynthesize organic matter (Visser *et al.* 2016). For example, Ji *et al.* (2020) reported that cell-density increased from 10^5 to 10^7 cells mL^{-1}, and pH values also increased above 8.0 (max. 9–10) for several months during a successive *Microcystis* bloom. These results suggested that these changes in cell-density and pH values of cyanobacteria-laden waters may strongly challenge water treatment processes in drinking water treatment plants (DWTPs).

In DWTPs, coagulation/sedimentation is a conventional drinking water treatment process to remove colloidal particles in source waters and it is also an important barrier to prevent the breakthrough of cyanobacterial cells. PACl is the most common coagulant, and it contains the stable preformed tridecameric polymer $Al_{13}O_4(OH)_{24}(H_2O)^{7+}$ referred to as Al_{13} polymer aluminium species that is thought to be more effective at charge neutralization than alum due to a higher charge density (Lin & Ika 2020). Previous studies have demonstrated that PACl coagulation could effectively remove cyanobacterial cells and algal organic matter (AOM) in varying degrees (Tang *et al.* 2017). However, cyanobacterial cells have average negative charge of −20 to −40 mW and AOM could react with PACl. These characteristics can result in a decrease of PACl coagulation efficiency and an increase of PACl demands (Tang *et al.* 2017). Over-use of PACl may also cause aluminium contamination in drinking water, leading to chronic toxicity to the human nervous system (Wang *et al.* 2019). Thus, investigating the effects of pH and cell-density on cyanobacterial removal is quite important for water supplies to optimize PACl application during a successive bloom.

The pre-chlorination process has been widely employed to treat cyanobacteria-laden source waters (Lin *et al.* 2018). It has been proven to be effective in promoting coagulation efficiency to remove cyanobacteria, since free chlorine can inactivate cyanobacterial cells via disrupting cell structures (Fan *et al.* 2013; Lin *et al.* 2018). However, some negative effects have also been reported, that chlorine can induce membrane damage, leading to the release of intracellular organic matter (IOM) (Lin *et al.* 2018). This released IOM may act as precursors to produce more disinfection by-products (DBPs) after chlorination, and it may hinder cyanobacterial removal by coagulation (Zamyadi *et al.* 2012; Zhou *et al.* 2014). Hence, during a successive bloom, whether the pre-chlorination process prior to coagulation is essential to treat cyanobacteria-laden resource waters was worthy of investigation.

To our knowledge, few studies have investigated water treatment processes to remove cyanobacteria during a successive bloom. In this study, we focused on typical characteristics of elevated cell-density and pH values of a successive bloom. PACl coagulation experiments were conducted to investigate cyanobacterial removal with varied pH and cell-density. Then, pre-chlorination prior to the coagulation process was performed to evaluate the overall removal ratio of cyanobacteria via measuring turbidity, chlorophyll-a, phycocyanin and dissolved organic carbon (DOC). This study aimed to provide an important reference for water supplies to choose proper water treatment processes to treat cyanobacteria-laden source waters during a successive bloom.

MATERIALS AND METHODS

Materials and reagents

Microcystis species has been one of the most common and problematic species (Harke *et al.* 2016), and thus, *Microcystis aeruginosa* FACHB-915 was employed for conducting the experiments. It was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences. The strain was cultured in BG11 medium at 28 °C with a 12 h:12 h light–dark cycle under light intensity of 35 μmol m^{-1} s^{-1} (Li *et al.* 2020a, 2020b). A culture volume of 1 L was employed with an inoculation volume of 1:10, and these cultures were shaken once at 10:00am on each day. After 14 d cultivation, the *Microcystis* samples were collected for subsequent experiments.

PACl used in this study was purchased from Tianjin Guangfu Fine Chemical Research Institute (Al_2O_3 content >28%, basicity 70%–75%). Sodium hypochlorite commercial solutions for the chlorination experiments were analytical grade (Sigma,

German). Moreover, all solutions were prepared using ultra-pure water purified to a resistivity of 18 MΩ cm by a Milli-Q water purification system (Millipore Pty Ltd, USA).

Coagulation experiments

Investigating the effects of pH values on cyanobacteria removal via PACl coagulation

Ohio EPA have selected chlorophyll-a thresholds of 2, 5, and 50 μg/L (or 4,000, 10,000, and 100,000 cells mL^{-1}) for minor, moderate, and severe blooms, respectively (Ohio EPA 2014). *Microcystis* cells were collected via centrifugation at 5,000 *g* for 10 min (extracellular organic matter was removed), resuspended with ddH_2O and achieved a final cell-density of 9.0×10^5 cells mL^{-1} characterized as severe blooms. During a severe *Microcystis* bloom, the pH value increased above 8.0 (max. 9–10), and it was about 7.0–8.0 without cyanobacterial bloom in natural freshwaters (Tang *et al.* 2018; Ji *et al.* 2020).

To investigate the effects of pH values on *Microcystis* removal by PACl coagulation, *Microcystis* samples were adjusted to pH of 7.5, 8.5, and 9.5 using either sodium hydroxide or hydrochloric acid, respectively. For coagulation experiments, 50 mL of cyanobacterial cultures were treated with various dosages of PACl (0, 2.5, 5, 10, 20, 30, 60, and 90 mg L^{-1}) in 200 mL glass conical flasks. Then, it was stirred continuously during the coagulation process (800 rpm min^{-1}, 1 min; 300 rpm min^{-1}, 4 min; 100 rpm min^{-1}, 15 min) with six magnetic agitators, and kept motionless for 40 min. Finally, *Microcystis* samples were taken for the measurements of turbidity, chlorophyll-a and phycocyanin. The details of the analytical methods are described below. These coagulation experiments were conducted at the same room-temperature (22 ± 2 °C).

Investigating the effects of cell-density on cyanobacteria removal via PACl coagulation

During a successive *Microcystis* bloom, cell-density ranged from 10^5 to 10^7 cells mL^{-1} for several months (Visser *et al.* 2016). To investigate the effect of cell-density on *Microcystis* removal by PACl coagulation, these collected *Microcystis* cells were resuspended with ddH_2O and achieved different cell-densities of about 9.0×10^5, 3.4×10^6, and 1.0×10^7 cells mL^{-1}, respectively. Cell-densities of *Microcystis* samples were measured by a flow cytometer (FlowSight, Merck Millipore, USA) (Li *et al.* 2020a). For coagulation experiments, 50 mL of cyanobacterial cultures were treated with various dosages of PACl (0, 2.5, 5, 10, 20, 30, 60, and 90 mg L^{-1}) in 200 mL glass conical flasks. Then, it was stirred continuously during the coagulation process (800 rpm min^{-1}, 1 min; 300 rpm min^{-1}, 4 min; 100 rpm min^{-1}, 15 min) with six magnetic agitators, and kept motionless for 40 min. Finally, *Microcystis* samples of surface water were taken for the measurements of turbidity, chlorophyll-a and phycocyanin, respectively. These coagulation experiments were conducted at the same room temperature (22 ± 2 °C).

Pre-chlorination plus coagulation experiments to remove cyanobacteria

Hypochlorite solutions were prepared from sodium hypochlorite (NaClO) commercial solutions. Free chlorine concentration was measured using the N,N,diethyl-p-phenylenediamine (DPD) method. *Microcystis* cells were collected, as described in the section 'Investigating the effects of pH values on cyanobacteria removal via PACl coagulation'. For pre-chlorination experiments, *Microcystis* samples of 50 mL were treated with various dosages of chlorine (0.5, 1, 2, 4, and 8 mg L^{-1}) in 200 mL glass conical flasks. After a contact time of 60 min, residual chlorine was quenched with sodium thiosulfate ($Na_2S_2O_3$) and these samples were taken for the measurement of turbidity, chlorophyll-a, phycocyanin, and dissolved organic matter (DOC), respectively. Then, various dosages of PACl (0, 2.5, 5, 10, 20, 30, 60, and 90 mg L^{-1}) were added to the *Microcystis* samples, as described above in the section 'Coagulation experiments'. After PACl coagulation, *Microcystis* samples were taken for the measurements of DOC, turbidity, chlorophyll-a and phycocyanin, respectively.

Analytical methods

Both *Microcystis* cells and algal organic matter (AOM) could contribute to the majority of turbidity in these samples, and thus, turbidity was employed to be the indicator of the removal of both *Microcystis* cells and AOM. Turbidity was measured with turbidometer (Orion AQ4500, USA). Chlorophyll-a and phycocyanin constituted the photosynthetic apparatus in *Microcystis* cells, and thus, both chlorophyll-a and phycocyanin were used as the parameters of *Microcystis* removal. The two parameters were measured using a two-channel fluorometer (AmiScience, USA). *Microcystis* samples were filtered with a 0.45 μm Millipore filter before the measurement of dissolved organic carbon (DOC) and DOC concentration was measured by the persulfate wet oxidation technique (Shimadzu TOC-V WP, Japan), as also described by Li *et al.* (2020a, 2020b). Cellular size of *Microcystis* was measured with a Bettersize2600E laser particle size distribution instrument (Jinke, China). Zeta potential of *Microcystis* was measured using a multi-angle particle size and high sensitivity zeta potential analyzer (NanoBrook Omni, USA).

Statistics analysis

Three parallel experiments were conducted. Differences of DOC, turbidity, chlorophyll-a and phycocyanin used Student's *t*-test, and were considered significant at $P < 0.05$. All statistical analyses were performed using Origin 8.0.

RESULTS

Effects of pH values on *Microcystis* removal via PACl coagulation

Figure 1 shows that PACl coagulation with initial dosages of 2.5–30 mg L^{-1} could effectively remove *Microcystis* at pH values of 7.5, 8.5 and 9.5. The removal ratio of turbidity, chlorophyll-a and phycocyanin at pH values of 8.5 and 9.5 was much higher than that at pH value of 7.5 with the equal initial dosage of PACl ($P < 0.05$), and there was no significant difference at pH values of between 8.5 and 9.5 ($P > 0.05$) (Figure 1). However, the removal ratio of *Microcystis* decreased with initial high dosages of 30, 60, and 90 mg L^{-1} (Figure 1).

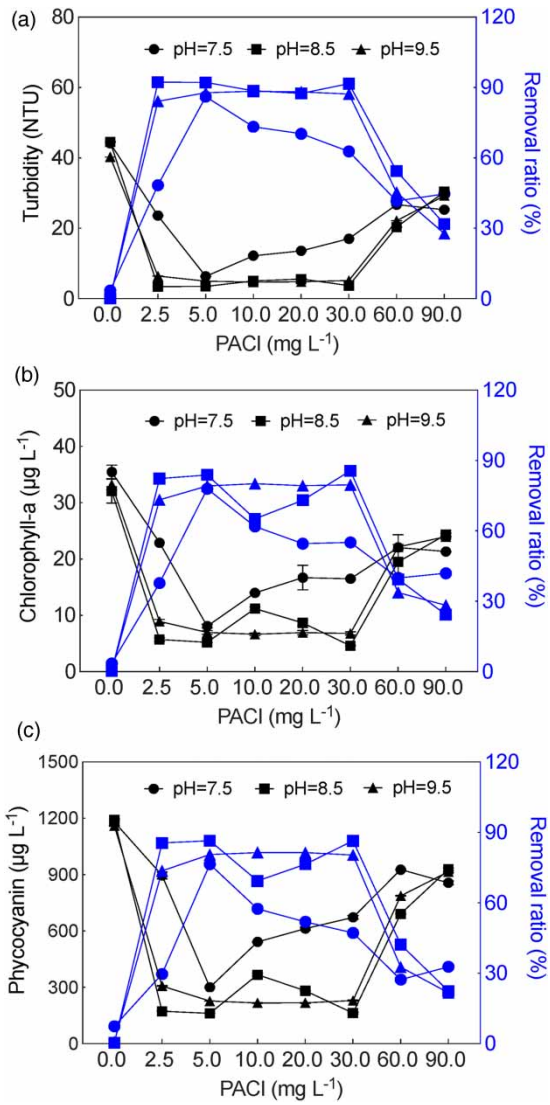


Figure 1 | Concentrations of (a) turbidity, (b) chlorophyll-a, (c) phycocyanin, and the corresponding removal ratio when *Microcystis* samples of 9.0×10^5 cells mL^{-1} were treated with PACl (0, 2.5, 5, 10, 20, 30, 60, and 90 mg L^{-1}) at pH values of 7.5, 8.5 and 9.5, respectively.

Effects of cell-density on *Microcystis* removal via PACl coagulation

During a successive *Microcystis* bloom, pH value increased above 8.0 (max. 9–10) for several months (Visser *et al.* 2016; Tang *et al.* 2018), and Figure 1 demonstrates that the removal ratio of *Microcystis* had no significant difference at pH values of between 8.5 and 9.5 ($P > 0.05$). Hence, subsequent coagulation experiments for *Microcystis* samples were conducted at a pH value of 9.5 (Figure 2).

Figure 2 shows that removal ratio of turbidity, chlorophyll-a and phycocyanin was dependent on the initial dosages of PACl, among which the removal ratio was higher for 3.4×10^6 and 1×10^7 cells mL^{-1} than that for 9×10^5 cells mL^{-1} with initial PACl dosages of 10–90 mg L^{-1} ($P < 0.05$) (Figure 2). However, the improved PACl coagulation could not be attributed to the high cell-density since the removal ratio for 9×10^5 cells L^{-1} reached up to about 90% with initial PACl dosage of 5 mg L^{-1} whereas the same treatment for 3.4×10^6 and 1×10^7 cells L^{-1} was less than 50% (Figure 2). Besides, with the initial PACl dosages of <10 mg L^{-1}, the removal ratio was much lower for high cell-densities of 3.4×10^6 and 1×10^7 cells mL^{-1} than that of 9×10^5 cells mL^{-1} ($P < 0.05$) (Figure 2).

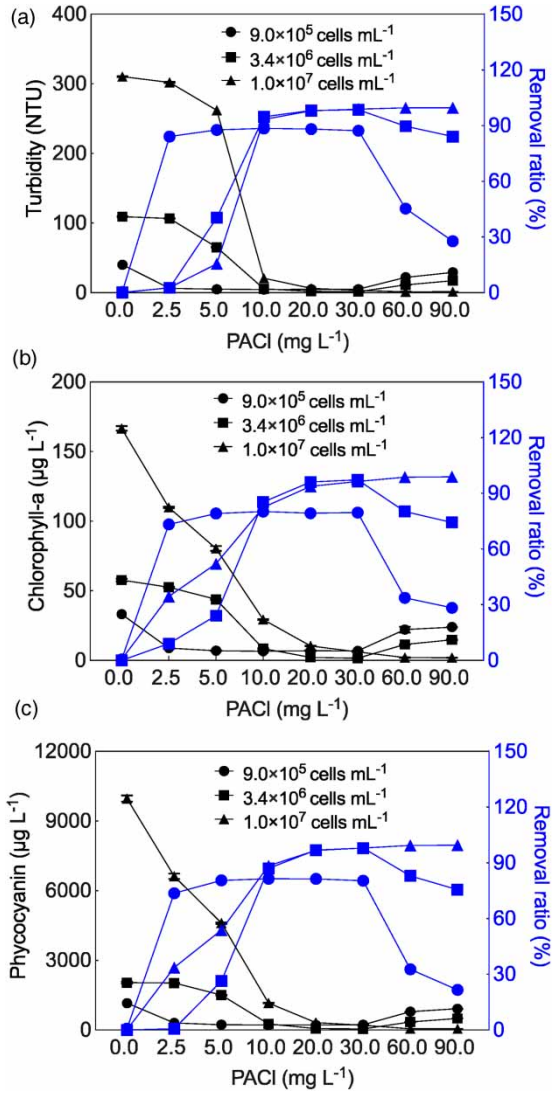


Figure 2 | Turbidity, chlorophyll-a, phycocyanin and the corresponding removal ratio when various cell-densities (9.0×10^5, 3.4×10^6, and 1.0×10^7 cells mL^{-1}) of *Microcystis* samples were treated with PACl (0, 2.5, 5, 10, 20, 30, 60, and 90 mg L^{-1}) at a pH value of 9.5 after a contact time of 60 min, respectively.

Pre-chlorination plus post-coagulation to remove *Microcystis*

The above study found that initial PACl of 5 mg L^{-1} only removed 3%–5% of *Microcystis* with a cell-density of 1.0×10^7 cells mL^{-1} (Figure 2). To enhance *Microcystis* removal, pre-chlorination of 0.5, 1, 2, 4 and 8 mg L^{-1} was employed to treat these *Microcystis* samples with high cell-density of 1.0×10^7 cells mL^{-1} at a pH value of 9.5 (Figure 3). Then, PACl coagulation of 5 mg L^{-1} was further conducted to remove *Microcystis* cells (Figure 4).

After pre-chlorination, both chlorophyll-a and phycocyanin showed a significant decrease of 30%–50% with initial high dosages of chlorine (2, 4 and 8 mg L^{-1}) whereas about 5%–10% increased with initial low dosages of chlorine (0.5 and 1 mg L^{-1}) (Figure 3). Meanwhile, both turbidity and DOC were increasing by 5%–10% in all treatments, but this was not dependent on initial dosages of chlorine (Figure 3).

PACl coagulation of 5 mg L^{-1} was further employed to treat chlorine-treated *Microcystis* samples (Figure 4). Figure 4 shows that the turbidity, DOC, chlorophyll-a and phycocyanin of *Microcystis* samples decreased after PACl coagulation in varied degrees. However, the removal ratio was chlorine-dosage-dependent, in which the highest removal ratio of 56.2% (turbidity), 41.6% (chlorophyll-a), 51.1% (phycocyanin) and 62.1% (DOC) was observed with pre-chlorination of 1 mg L^{-1} whereas the initial highest dosage of pre-chlorination (8 mg L^{-1}) achieved the lowest removal ratio of *Microcystis* cells and DOC (Figure 4).

A comparison of PACl coagulation alone and pre-chlorination plus coagulation to remove *Microcystis*

To further compare the removal efficiency of *Microcystis* (1×10^7 cells mL^{-1}) by two treatment processes (PACl coagulation; chlorination-assisted PACl coagulation), the increasing ratio (IR) of the removal ratio of DOC, turbidity, chlorophyll-a and phycocyanin of the two treatment processes was estimated by Equation (1):

$$\mathrm{IR}\ \% = 100 \times (\eta_2 - \eta_1)/\eta_1 \tag{1}$$

where IR % is the increasing ratio of the removal ratio of DOC, turbidity, chlorophyll-a and phycocyanin; η_1 is the removal ratio of DOC, turbidity, chlorophyll-a and phycocyanin with the treatment of PACl coagulation alone (5 mg L^{-1}); and η_2 is the

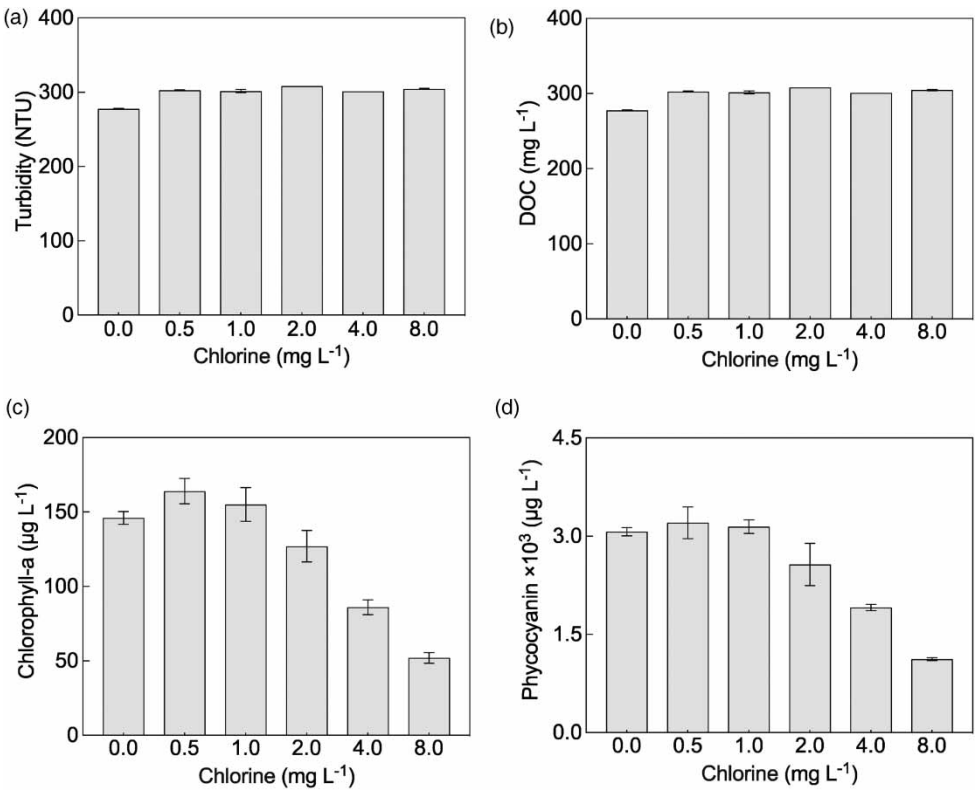


Figure 3 | Concentrations of (a) turbidity, (b) DOC, (c) chlorophyll-a and (d) phycocyanin when *Microcystis* samples of 1×10^7 cells mL^{-1} (pH 9.5) were treated via the pre-chlorination process with various doses of 0.5, 1, 2, 4, and 8 mg L^{-1} after a contact time of 60 min, respectively.

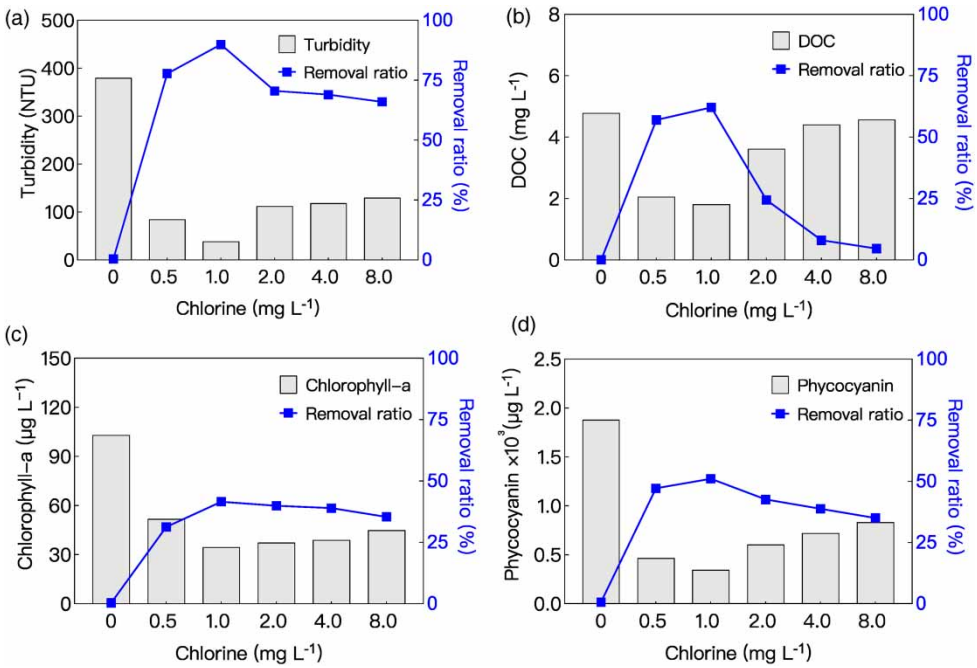


Figure 4 | Concentrations of (a) turbidity, (b) DOC, (c) chlorophyll-a and (d) phycocyanin after PACl coagulation of 5 mg L^{-1} to remove chlorine-treated *Microcystis* of 1×10^7 cells mL^{-1} with various dosages of 0.5, 1, 2, 4, and 8 mg L^{-1} at a pH value of 9.5, respectively.

removal ratio of DOC, turbidity, chlorophyll-a and phycocyanin with the treatment of chlorination-assisted (1 mg L^{-1}) PACl coagulation (5 mg L^{-1}).

The data of η_1 and η_2 were gained from Figures 3 and 4, respectively. Figure 5 shows that chlorination-assisted PACl coagulation could achieve RI (about 200%, 400% and 800%) of turbidity, chlorophyll-a and phycocyanin, respectively. This suggested that the pre-chlorination process could improve the removal efficiency of high cell-density *Microcystis* by PACl coagulation. However, IR of DOC ranged from about 50% to −100%, suggesting that the DOC removal was dependent on initial chlorine dosage (Figure 5). Only low dosages of chlorine (0.5 and 1 mg L^{-1}) promoted DOC removal, while higher dosages resulted in elevated DOC even after PACl coagulation (Figure 5).

DISCUSSION

Effects of pH values on *Microcystis* removal via PACl coagulation

Previous studies have noted that pH values played an important role in PACl coagulation, since hydrolysis products of PACl would be varied in the range of 1–10 (Naceradska *et al.* 2019). Wu *et al.* (2020) found that the highest coagulation efficiency

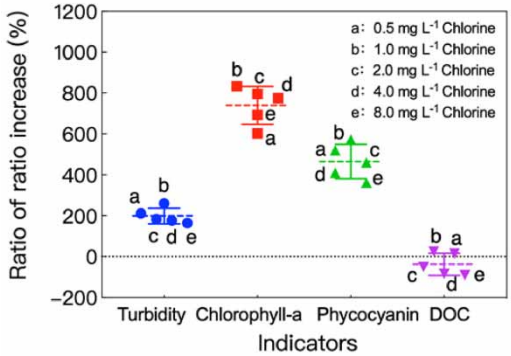


Figure 5 | Increasing ratio of removal ratio of turbidity, chlorophyll-a, phycocyanin and DOC when two treatment processes (PACl coagulation alone; chlorination-assisted PACl coagulation) were employed to treat a high cell-density of cyanobacteria (1×10^7 cells mL^{-1}).

occurred at pH values of 7–8. This may be explained by the hydrolysis form of PACl being mainly comprised of cationic polymers (e.g., $Al_{13}(OH)_{34}^{+5}$) at pH values of 7–8, and these cationic polymers contribute to cyanobacteria removal via electrical neutralization (Lin & Ika 2020). However, this study found that elevated pH values of 8.5–9.5 gained higher removal ratios of *Microcystis* than the pH value of 7.5. Actually, at pH value >8.0, the hydrolysis form of PACl mainly consists of $[Al(OH)_4^-]$ and *Microcystis* cells are also negative (Lin & Ika 2020). This result suggested that electrical neutralization might not be the key mechanism to enhance *Microcystis* removal by PACl coagulation at pH values of 8.5–9.5.

Tian & Zhao (2021) proposed that the polymerization degree of aluminium salt increases and that it forms a complex structure of hydroxyl polymer at pH value of >8.0. Moreover, a hydrolytic polymerization reaction tends to an increase of polymerization degree, and thus, polymerized aluminium hydroxyl complex ions easily form and eventually change into neutral gelatinous precipitate (Yang 2013). These gelatinous precipitates may act as a net to capture and sweep cells to enhance *Microcystis* removal at pH values of 8.5–9.5. Overall, during a successive bloom, PACl coagulation to remove cyanobacteria can benefit from the *in situ* elevated pH of the source waters.

Effect of cell-density on *Microcystis* removal via PACl coagulation

To our knowledge, there is limited literature to investigate the effects of cell-density on *Microcystis* removal via PACl coagulation during a successive bloom. Henderson *et al.* (2010) demonstrated that cellular surface area and charge density held a strong positive correlation with coagulant demands. In this study, elevated cell-density of *Microcystis* exhibited larger total cellular surface area and total charge density, and thus, PACl coagulation efficiency decreased with elevated cyanobacterial biomass. Hence, during a successive bloom, cyanobacterial biomass can be a striking factor to decrease PACl coagulation.

Chlorination-assisted PACl coagulation to remove *Microcystis*

After pre-chlorination, elevated DOC and turbidity indicated that chlorine had disrupted the cellular membrane, leading to the release of intracellular organic matter (IOM) (Figure 3; Supplementary Information, Figure S1). A similar result has also been documented by previous studies (Zamyadi *et al.* 2012; Zhou *et al.* 2014). Notably, elevated DOC/turbidity had no significantly positive correlation with initial dosages of chlorination, and a higher initial dosage of chlorine (2, 4, and 8 mg L^{-1}) did not result in a higher DOC concentration (Figure 3). This result suggested that the released IOM may be oxidized into CO_2 after dosing with sufficient chlorine exposure.

This study found that pre-chlorination enhanced *Microcystis* removal by PACl coagulation (Figures 4 and 5), in agreement with previous studies (Xie *et al.* 2016). However, associated mechanisms may differ with initial dosages of chlorine. Initial low dosages of chlorine (0.5 and 1 mg L^{-1}) did not inactivate *Microcystis* due to a slight increase of chlorophyll-a and phycocyanin (Figure 3). Ma *et al.* (2012) noted that a high molecular weight of AOM aided coagulation by favoring the formation of larger flocs. In these treatments with chlorine (0.5 and 1 mg L^{-1}), the released high molecular weight (MW) of IOM could be the main mechanism to enhance *Microcystis* removal. In contrast, higher dosages of chlorine (2, 4 and 8 mg L^{-1}) effectively inactivated *Microcystis* via destroying chlorophyll-a and phycocyanin (Figure 3). This result suggested that the activation of *Microcystis* could be the main mechanism to enhance *Microcystis* removal by PACl coagulation.

Moreover, after PACl post-coagulation, *Microcystis* removal was not dependent on initial chlorine dosage, in which initial high dosage of chlorine (2, 4, and 8 mg L^{-1}) exhibited a lower removal ratio of *Microcystis* and DOC than initial dosages of 0.5 and 1 mg L^{-1} ($P < 0.05$) (Figure 4). Safarikova *et al.* (2013) found that low-MW proteins exhibited stronger inhibitory effects on the coagulation process than high-MW proteins. This suggested that the possible formation of low-MW organic matter after chlorination (2, 4, and 8 mg L^{-1}) strongly decreases the removal of *Microcystis*. Meanwhile, this low-MW organic matter was also difficult to remove by PACl coagulation, leading to an increase of DOC. In contrast, initial low dosage of chlorine (0.5 and 1 mg l^{-1}) mainly induced the release of high-MW IOM, and this IOM could not be oxidized into low-MW IOM due to insufficient chlorine exposure. This high-MW IOM aided *Microcystis* removal, and was easily removed by PACl coagulation, leading to an decrease of DOC. Moreover, the removal ratio of *Microcystis* that occurred with pre-chlorination (0.5 mg L^{-1}) was lower than that with the dosage of 1.0 mg L^{-1}, possibly attributable to its lower amounts of released high-MW IOM. Overall, moderate pre-chlorination (1 mg L^{-1}) could be a promising option to enhance the removal of high cell-density *Microcystis* (10^7 cells mL^{-1}) by PACl coagulation.

Practical implication

The concept of 'a successive bloom' is essential for water supplies to determine proper water treatments to treat cyanobacteria-laden source waters, but it was always ignored by previous studies. During a successive *Microcystis* bloom, changes

in cell-density and pH value were significant characteristics of source waters. This study suggested that *Microcystis* removal by PACl coagulation could benefit from *in situ* elevated pH (>8.5) of source waters. However, elevated cyanobacterial biomass strongly hindered PACl coagulation and the inhibitory effects on PACl coagulation could not be offset by *in situ* elevated pH value. These results demonstrated that cyanobacterial biomass could be a more important factor to affect PACl coagulation than elevated pH value. Thus, it is quite important for water supplies to monitor cyanobacterial biomass in real-time to determine the effective drinking water treatment process during a successive bloom.

To our knowledge, whether pre-chlorination was essential to treat cyanobacteria-laden source waters was a heated argument, attributable to its apparent advantages and drawbacks (e.g., severe membrane destruction). During a successive bloom, this study proposed that the application of pre-chlorination was mainly dependent on cyanobacterial biomass. For high cell-density of $>10^7$ cells mL^{-1}, moderate pre-chlorination (1 mg L^{-1}) was essential to promote PACl coagulation efficiency to remove *Microcystis*. For low cell-density of 10^5–10^6 cells mL^{-1}, coagulation alone was sufficient to remove *Microcystis*, since it can benefit from *in situ* elevated pH value of source waters. These results provide a useful reference for water supplies to choose the proper water treatment process to treat cyanobacteria-laden source waters during a successive bloom.

Although this study demonstrated that moderate chlorination (1 mg L^{-1}) eliminated the risk of elevated DOC after PACl coagulation, other undesirable metabolites (e.g., cyanotoxins; taste/odor compounds) would be released after cell rupture (Zamyadi *et al.* 2012; Fan *et al.* 2013; Li *et al.* 2020a, 2020b). Notably, disinfection by-products would be another troubling problem after chlorination, since previous studies found that AOM could be important precursors to form DBPs after chlorination (Zamyadi *et al.* 2012; Zhou *et al.* 2014; Wu *et al.* 2019). Consequently, more studies are required to systematically assess the advantages and drawbacks of moderate chlorination-assisted PACl coagulation before it is employed to remove a high cell-density of *Microcystis*.

CONCLUSIONS

During a successive bloom, *Microcystis* removal via PACl coagulation can benefit from *in situ* elevated pH (>8.5), and thus, coagulation alone is recommended to treat cyanobacteria-laden source waters with low cell-density of 10^5–10^6 cells mL^{-1}. However, elevated cyanobacterial biomass was a striking factor in decreasing cyanobacterial removal by PACl coagulation, since its inhibitory effects on coagulation could not be offset by *in situ* elevated pH value. Moderate chlorination-assisted (1 mg L^{-1}) coagulation is recommended to treat cyanobacteria-laden source waters with high cell-density of $>10^7$ cells mL^{-1}, but more studies should be done to assess the advantages and drawbacks for removing cyanobacteria with high cell-density in the future.

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AUTHOR STATEMENTS

Weijun Song: Investigation, Writing/revising manuscript.
Yu Xie: Data Curation, Revising manuscript.
Xunfang Wu: Investigation, Revising manuscript.
Jie Zeng: Revising manuscript, Polishing language.
Xi Li: Experimental design, Writing/revising manuscript, Conceptualization, Project management.

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

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